B-mode and Doppler Ultrasonography of Accessory Genital Glands and Testes in Male Goats During the Breeding Season

*Hamed Talaat Elbaz, Ahmed Mohamed Sharshar and Ahmed Essam Elweza**

Abstract

The aims of the present study were to scan the echogenicity of reproductive organs of bucks during the breeding season. The influence of testosterone on haemodynamic Doppler indices of accessory genital glands of breeding bucks was also examined. Ten clinically healthy, sexually mature, Egyptian Baladi male goats were examined and the testes, tail of epididymis and accessory sex glands imaged using greyscale B-mode, colour Doppler ultrasonography. The spectral Doppler indices (pulsatility index and resistive index) were measured. Blood samples were collected and serum concentrations of testosterone, FSH and LH were determined. The results revealed that the echogenicity of testes, tail of epididymis and accessory genital glands was changed by breeding season. Pulsatility index values of supra-testicular artery, marginal artery, tail of the epididymis, ampulla, vesicular gland, pars disseminata of the prostate and bulbourethral gland were 0.85±0.04, 0.54±0.03, 0.4±0.03, 0.37±0.04, 0.51±0.03, 0.39±0.02 and 0.41±0.04, respectively. The resistive index of the above criteria were 0.51±0.04, 0.37±0.02, 0.3±0.03, 0.27±0.02, 0.31±0.03, 0.32±0.03 and 0.32±0.03, respectively. Serum testosterone concentration was 4.78±0.46 ng/mL. Furthermore, FSH and LH were 3.71±0.43 and 1.8±0.17 mIU/mL, respectively. Interestingly, testosterone clearly deceased the values of the both the pulsatility and resistive indices of the accessory genital glands of breeding bucks. Season modified the echogenicity of testes, epididymis and accessory genital glands. Testosterone regulated the reproductive indices of blood flow of the accessory genital glands of breeding bucks. Thus, this study could serve as a baseline of reference values of Egyptian Baladi male goats during the breeding season to improve reproductive efficiency.

Key words: *doppler; season; testes; genital glands; breeding bucks*

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Introduction

Breeding bucks are a substantial investment and breeding soundness examinations of bucks are conducted to judge their fertilisation capabilities (Ridler et al., 2012). Colour Doppler ultrasonography has recently been introduced as a diagnostic tool in veterinary medicine for the evaluation of male reproductive performance in domestic animals (Ginther, 2014). It has been applied as an unconventional diagnostic procedure in male camels (Kutzler et al., 2011), stallions (Pozor, 2007), dogs (Polisca et al., 2013) and rams (Camela et al., 2017). Doppler ultrasonography of the testicular artery was used in veterinary practice to determine the normal function of testes in dogs (Zelli et al., 2013) and stallions (Pozor and McDonnell, 2004; Pozor, 2007). Seasonal changes in daylight and ambient temperature modulates the haemodynamic parameters of the testicular artery in male Shiba goats (Samir et al., 2018). Besides, Doppler indices like the resistive index (RI) and pulsatility index (PI) of the testicular artery were recorded with maximum values for predicting testicular function and semen quality in rams (Hedia et al., 2019). In goats, real time ultrasonographic scanning was used as an adequate practical procedure for diagnosis of early pregnancy (Samir et al., 2016). The use of colour Doppler ultrasonography in the reproduction of small ruminants is infrequent, particularly for males (Serine et al., 2010; Samir et al., 2015), and the literature on studies of male reproduction with colour Doppler ultrasonography in small ruminants is scarce (Serine et al., 2010; Samir et al., 2015; Elbaz et al., 2019).

Accessory sex glands participate significantly in male fertility, though unfortunately they have not been sufficiently considered during the breeding soundness evaluation (BSE) of small ruminants (Gouletsou and Fthenakis, 2010). Application of trans-rectal ultrasonography for visualization of changes in the accessory sex glands in bucks is still infrequent (Elbaz and Abdel Razek, 2019). Seminal fluids secreted from the accessory sex glands in bucks consist of complex nutrients essential for peak motility and fertilizing capacity of sperm (Gofur, 2015). Regarding bucks, sexual examinations should be carried out 2 months prior to the breeding season to assure ideal reproductive performance (Tibary et al., 2018). Recently, our group showed that the treatment of bucks with GnRH increased serum testosterone level and enhanced the dimensions of the accessory sex glands during the non-breeding season (Elbaz et al., 2019). To the best of our knowledge, there are no available data concerning monitoring of the accessory genital glands of bucks using colour Doppler ultrasound during the breeding season. Therefore, the objectives of the present study were to scan scrotal contents and accessory genital glands in Egyptian Baladi male goat using B-mode and colour Doppler ultrasonography during the breeding season. Moreover, the influence of testosterone on the reference values of PI and RI of the accessory genital gland of breeding bucks was examined.

Materials and methods

Ethics approval

This present study received Ethics approval from the Animal Care and Welfare Committee, University of Sadat City, Egypt.

Animals

Ten sexually mature Egyptian Baladi male goats, aged from 1.5 to 2 years and weighing 40 to 50 kg were included in the study. Animals were housed at the educational farm of the Faculty of Veterinary Medicine, University of Sadat City, Egypt, during the period from October to December 2019. Male goats were healthy, regularly dewormed, vaccinated, and usually kept free in a stall barn, and fed with pellet concentrates and tibn, with free access to the water and green fodder (barseem clover). Comprehensive breeding soundness examinations were carried out on all bucks to ascertain they were free from any reproductive disorders. The examined animals were separated from females throughout the study.

Ultrasonographic examination of the scrotum and its contents

Animals were restrained mechanically and scanned twice weekly throughout the study period. Ultrasound examinations of animals were carried out by using linear and micro-convex probes of the scanner (2.5-22 MHz; Esaote MyLab™One VET, Italy). Ultrasonographic imaging of scrotal contents was performed in the standing position. Hairs on sides of the scrotum were cleaned and shaved carefully. Both Gray scales (B-mode) and colour Doppler ultrasonographic examinations were performed using the same methods described elsewhere (Gouletsou et al., 2003; Samir et al., 2015; Elbaz et al., 2019).

Colour Doppler ultrasound was initially positioned at the scrotum neck to scan the supra-testicular artery at the convoluted looping section. Then, to localize the marginal artery and the intra-testicular arteries, the transducer was moved downward over the testicular parenchyma. Colour gain was examined and an electronic calliper (2–3 mm in length) was positioned at the central area of the vascular vessel with apertures to detect the spectral curve, haemodynamic indices and spectral trace of vascular flow. The apparatus software was set to estimate the resistive index (RI) and pulsatility index (PI). The Doppler gate was continually reserved at 1 mm and at least three spectral curves were obtained (Pozor and McDonnell, 2004; Samir et al., 2018). All examinations were conducted by the same individual at the same time with fixed ultrasound settings.

Doppler ultrasound scanning of the accessory genital glands

A self-manufactured connector was fixed to the transducer to simplify its handling per rectum. The transducer was lubricated with coupling gel, inserted into the rectum after faecal evacuation, and then moved anteriorly to locate the guide (urinary bladder-pelvic urethral connection). The ampulla and vesicular glands were examined near the urinary bladder. The pars disseminate of the prostate gland was visualized during scanning of the pelvic urethra. During retraction of the probe from the anus, the bulbourethral glands were located and examined as previously mentioned by Camela et al. (2017).

Blood sampling

After ultrasonographic scanning, blood samples (5 mL) were collected from the jugular vein of all bucks. Sample were placed in a tube at 4°C for 12 h. Clotted blood was centrifuged at 3000 rpm for 15 minutes and separated sera were stored at -20°C in Eppendorf tubes until further analysis. Serum level of hormones (testosterone, FSH and LH) was measured with the ELISA kit (Calbiotech, Austin, USA) according to manufacturer instructions, using the microwell method and the optical density (OD) absorbance has been adjusted at 450 ±10 nm (Elbaz et al., 2019).

Statistical analysis

All data are presented as means ± standard error of the mean (SEM). Statistical analysis was conducted using

GraphPad Prism 5 software (La Jolla, CA, USA). Values of $P<0.05$ were considered statistically significant. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to examine the effect of testosterone on the pulsatile index (PI) and resistance index (RI) of the accessory genital glands.

Results

B-mode and Colour Doppler ultrasonographic imaging of the scrotum and its contents

Longitudinal image (B-mode) of the testis showed a moderately hypoechogenic testicular parenchyma (TP). The mediastinum testis (MT) appeared as a high white echogenic line in the middle of testis in longitudinal section. Scrotal skin (SS) and tunica albuginea (TA) appeared as hyperechogenic structures covering the testis (Fig. 1). The colour Doppler image of the testis showed small colour dots of the marginal artery (MA) appeared at the periphery, with intra-testicular arteries distributed at the testicular parenchyma (Fig. 2). The tail of the epididymis was imaged in the oblique plane and appeared as a non-echogenic triangular structure near the distal pole of the testis. By colour Doppler scanning, small red dots were imaged between the testicular parenchyma and tail of the epididymis from medial aspects of testis (Fig. 3). The pampiniform plexus appeared as non-echogenic area containing hyperechogenic scattered spots like a network on the upper pole of testis. Colour Doppler scanning showed the pampiniform plexus as different, large, colour

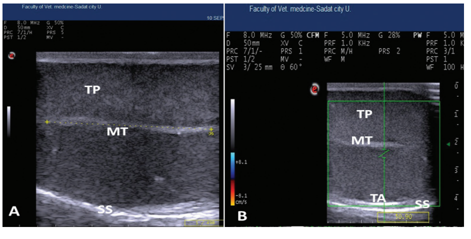

Figure 1. Ultrasonographic imaging of testicular parenchyma (TP). A) B- mode B) colour doppler ultrasonography showing the moderate hypoechogenicity of the testicular parenchyma (TP), echogenic central mediastinum testis (MT), more echogenic white tunica albuginea (TA) and scrotal skin (SS)

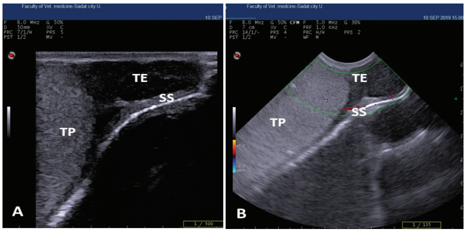

Figure 3. Ultrasonography of the epididymal tail. A) B-mode showing the non-echogenic black lumen of the tail of epididymis (TE), testicular parenchyma (TP) and scrotal skin (SS). Echogenic white colour of the scrotal skin (SS). B) Colour doppler image showing small coloured dots at the distal part of the epididymal tail

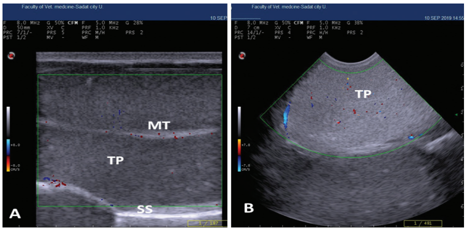

Figure 2. Colour Doppler ultrasonography of the testes. A), B) showing colours of the marginal and intra-testicular arteries. Testicular parenchyma (TP), echogenic mediastinum testis (MT) and scrotal skin (SS)

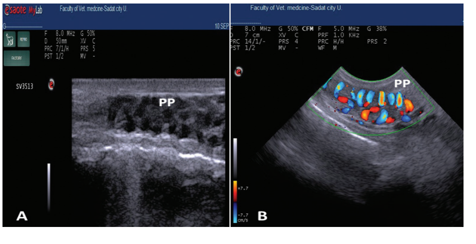

Figure 4. Ultrasonographic imaging of the spermatic cord. (A) B-mode of the pampiniform plexus (PP), and (B) colour Doppler showing blood flow within the convoluted part of the supratesticular artery in the pampiniform plexus (PP)

Table 1. Doppler parameters (PI and RI) of testes and tail of epididymis of male goats. (STA) supra-testicular artery, (MA) marginal artery, (RI) resistive index, (PI) pulsatility index. Data are presented as (mean ± SEM)

Testes				Tail of epididymis	
STA		MA			
PI	RI	PI	RI	PI	RI
0.85±0.04	0.51±0.04	0.54±0.03	0.37±0.02	0.4±0.03	0.3±0.03

patches varying from red to blue along the region of the spermatic cord (Fig. 4). The data of the spectral doppler indices (PI & RI) of blood flow of testis and tail of epididymis are presented in Table 1.

B-mode and Colour Doppler ultrasonographic imaging of accessory genital gland and pelvic urethra

During transrectal ultrasonographic examination of bucks, the ampullae were located dorsal to the bladder and appeared as a non-echogenic lumen and echogenic glandular lining (Fig. 5). Vesicular glands were examined near the urinary bladder and appeared irregular in shape, with hypoechogenic glandular tissue and non-echogenic rim at the centre of the glandular parenchyma. Colour Doppler showed a very small red dot in the ventral aspect of the gland (Fig. 6). The prostate gland (pars disseminata) appeared as an echogenic area between the hypoechogenic urethral muscle and the non-echogenic urethral lumen. Colour Doppler of the pars disseminata showed a red dot near the dorsal and ventral part of lumen (Fig. 7). The bulbourethral glands appeared nearly circular with non-echogenic parenchyma, interspersed with white streaks. Colour Doppler of the gland showed a single coloured dot in the

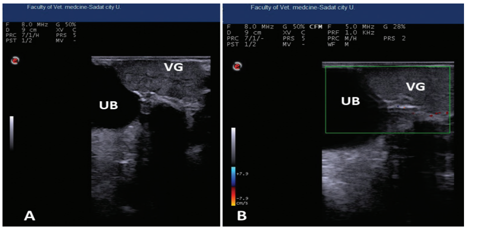

Figure 6. Ultrasonographic imaging of the vesicular gland. A) B-mode showing large pyriform with moderate echogenicity, lower circumscribed echogenic line and black non-echogenic urinary bladder (UB). B) Colour Doppler showing a tiny red dot in the ventral aspect of the vesicular gland (VG)

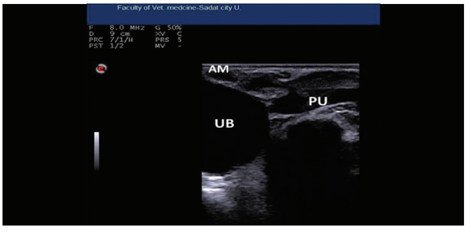

Figure 5. Ultrasonographic imaging of ampulla (AM). Note the small black non-echogenic lumen of ampulla (AM) and the non-echogenic black urinary bladder (UB) connected to the pelvic urethra (PU)

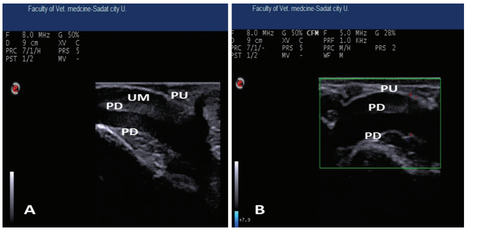

Figure 7. Ultrasonographic imaging of the pars disseminata of the prostate gland. A) B-mode showing the echogenic part of the pars disseminata (PD) surrounding the lumen of the pelvic urethra (PU) and urethral muscle (UM). B) Colour Doppler showing a red dot near the dorsal and ventral parts of the lumen

dorsal and ventral aspect (Fig. 8). Colour Doppler of pelvic urethra revealed tiny red dots distributed along the dorsal and ventral aspects of the urethra (Fig. 9). The data of spectral doppler indices (PI & RI) of blood flow of accessory genital glands are presented in Table 2.

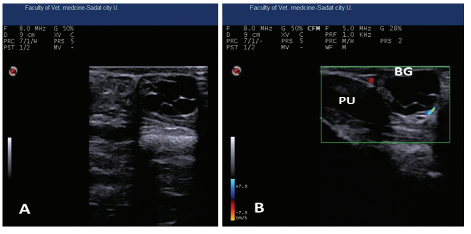

Figure 8. Ultrasonographic imaging of the bulbourethral glands (BG). A) B-mode showing the non-echogenic and white streaks of parenchyma of the gland. B) Colour Doppler showing a single, coloured dot in the dorsal and ventral aspect

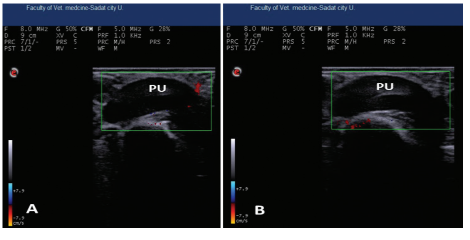

Figure 9. Colour Doppler ultrasonography of the pelvic urethra (PU). A) and B) Colour doppler ultrasonography showing the black, non-echogenic lumen surrounded by hypoechogenic urethral muscle (UM) and tiny red dots distributed along the dorsal and ventral aspects of the urethra

Serum hormonal concentrations

The serum testosterone concentration was 4.78±0.46 ng/mL. Furthermore, FSH and LH were 3.71±0.43 and 1.8±0.17 mIU/mL, respectively.

Influence of testosterone on Doppler parameters of accessory genital glands

Circulating testosterone highly reduced the haemodynamic parameters (PI and RI) of the ampulla, vesicular gland, prostate gland and bulbourethral gland (Figs. 10 and 11).

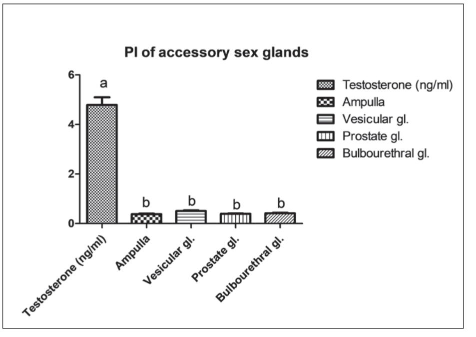

Figure 10. Effect of testosterone on the pulsatility index (PI) of the accessory genital glands of breeding bucks (n=10). Different superscript letters denote a significant difference at $P<0.05$. Data are presented as (mean ± SEM)

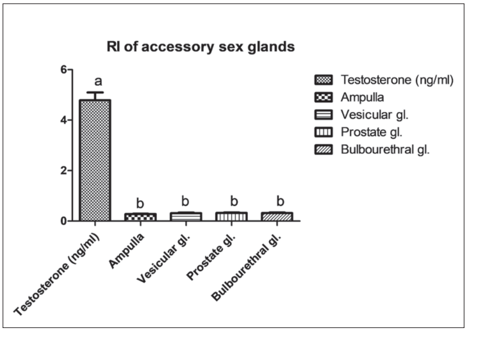

Figure 11. Effect of testosterone on the resistive index (RI) of accessory sex glands of breeding bucks (n=10). Different superscript letters denote a significant difference at $P<0.05$. Data are presented as (mean ± SEM)

Table 2. Doppler parameters, pulsatility index (PI) and resistive index (RI) of the accessory sex glands (ampulla, vesicular, prostate, bulbourethral) of male goats. Data are presented as (mean ± SEM)

Ampulla		Vesicular gland		Prostate gland		Bulbourethral gland	
PI	RI	PI	RI	PI	RI	PI	RI
0.37±0.04	0.27±0.02	0.51±0.03	0.31±0.03	0.39±0.02	0.32±0.03	0.41±0.04	0.32±0.03

Discussion

Seasonal changes in daylight and ambient temperature modulated haemodynamic parameters of the testes and circulating oestradiol in male Shiba goats (Samir et al., 2018). Goats are frequently described as seasonal breeders with their sexual activity mostly dependent on geographical position and breed (Fatet et al., 2011). The data presented here provide guidelines for colour Doppler ultrasonography of the testes and accessory genital glands of bucks during the breeding season.

In the present study, the testes showed moderate hypo-echogenicity of the testicular parenchyma, in agreement with results obtained by Ahmad et al. (1991) for sheep and goats. Nevertheless, a recent study by Elbaz and Abdel Razek (2019) during the non-breeding season described the testicular parenchyma of bucks as varying from low to moderate echogenicity. Colour Doppler imaging of the testes showed small coloured dots of the marginal artery at the periphery, and intra-testicular arteries distributed in the testicular parenchyma. These results were in accordance with previous studies in rams (Samir et al., 2015; Elbaz et al., 2019), dogs (Zelli et al., 2013) and stallion (Pozor and Mcdonnell, 2004). In the present study, the tail of the epididymis appeared with B-mode ultrasonography as triangular with a non-echogenic black colour, in line with results obtained by Ahmad et al. (1991). In contrary, the epididymal tail appeared heterogeneous in echogenicity during the non-breeding season (Elbaz and Abdel Razek, 2019). This difference might be attributed to season, testosterone level, relative humidity or photoperiod. Colour Doppler scanning of the epididymal tail appeared as small red dots between the testicular parenchyma and tail from the medial aspects, in agreement with results in Barki rams (Elbaz et al., 2019). The pampiniform plexus appeared as a non-echogenic area containing hyperechogenic scattered spots like a network on the upper pole of testis, in accordance with previously published data (Elbaz and Abdel Razek, 2019). Colour Doppler scanning of the pampiniform plexus appeared as large colour patches, varying from red to blue, along the region of spermatic cord. Spectral doppler ultrasonographic monitoring of pampiniform plexus was a monophasic blood flow pattern of low resistance, in agreement with previous results in rams (Elbaz et al., 2019).

The Doppler indices represented by the pulsatility index (PI) and resistance index (RI) could measure the resistance of blood flow peripheral to the inspected vessel (Serine et al., 2010). In the present study, values of PI and RI of the supra-testicular artery (STA) in the pampiniform plexus, marginal artery (MA) and tail of epididymis ranged from 0.3±0.03 to 0.85±0.04, in line with published data for bucks and rams (Samir et al., 2018; Elbaz et al., 2019). Here, we report that testosterone decreased the PI and RI values of ampulla, vesicular gland, pars disseminata and bulbourethral gland, suggesting that testosterone regulates vascular supply of blood to the accessory genital glands and therefore their secretory activities in breeding bucks. Supporting our data, the increase of blood flow was found to decrease the values of PI and RI in order to supply nutrients and oxygen to the corresponding organs (Varughese et al., 2013). Recently, a significant negative correlation was reported between PI, RI and active progressive sperm motility (Hedia et al., 2019).

In reviewing the available literature, this is the first study to report the colour Doppler ultrasonographic scanning of accessory genital glands and pelvic urethra of mature bucks during the breeding season. The ampulla appeared as a non-echogenic lumen and echogenic glandular lining, while the gland appeared as a hypoechogenic small lumen in the non-breeding season (Elbaz and Abdel Razek, 2019). The vesicular gland appeared irregular in shape with hypoechogenic glandular tissue and a non-echogenic rim in the centre of the glandular parenchyma. However, the echogenicity of glands was hypoechogenic to less echogenic in summer (Elbaz and Abdel Razek, 2019). The echogenicity of vesicular glands changed according to fluid accumulation (Chandolia et al., 1997). The prostate gland (pars disseminata) appeared as an echogenic area between the hypoechogenic urethral muscle and the non-echogenic urethral lumen. However, the gland appeared hyperechoic during summer (Elbaz and Abdel Razek, 2019). The colour Doppler of the pars disseminata showed a red dot near the dorsal and ventral parts of the lumen, which is in accordance with data of rams (Camela et al., 2017). The bulbourethral glands appeared nearly circular with a non-echogenic parenchyma and interspersed with white streaks. However, during the non-breeding season, the bulbourethral gland appeared with variable echogenicity from hypo-echogenic to moderate echogenicity (Elbaz and Abdel Razek, 2019). Colour Doppler of the gland showed a single, coloured dot in the dorsal and ventral aspect, and similar results were obtained in rams (Camela et al., 2017).

Colour Doppler of the pelvic urethra revealed mini red dots distributed along the dorsal and ventral aspects of the urethra. The current data are in line with the results of rams (Camela et al., 2017). The obtained results revealed that spectral Doppler ultrasonographic monitoring of the accessory genital glands showed a monophasic blood flow pattern of low resistance, in accordance with results obtained by Camela et al. (2017). The serum testosterone level of Egyptian Baladi breeding bucks in the current study was 4.78±0.46 ng/mL. On the contrary, a recent study in bucks during the non-breeding season showed a low level of testosterone, at 1.84±0.18 ng/mL (Elbaz and Abdel Razek, 2019). This difference could be attributed to the season effect, relative humidity, photoperiod, nutrition or sexual activity.

Conclusion

The breeding season modified the echogenicity of testes, tail of epididymis and accessory genital glands. Testosterone regulated the haemodynamic parameters of the ampulla, vesicular gland, prostate gland and bulbourethral gland of breeding bucks. Further studies are need to clarify the impact of haemodynamics of the genital organs on fertility and reproductive efficiency of bucks.

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Ultrazvuk B-moda i doppler ultrazvuk akcesornih spolnih žlijezda i testisa jaraca tijekom sezone parenja

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Cilj je ove studije bio skenirati ehogenost spolnih organa jaraca tijekom sezone parenja, a ispitan je i učinak testosterona na hemodinamičke doppler indekse aksecornih spolnih žlijezda rasplodnih mužjaka. U deset klinički zdravih, spolno zrelih egipatskih baladi jaraca ispitani su testisi, rep epididimisa i akcesorne spolne žlijezde putem ultrazvuka B-moda sive skale i color doppler ultrazvuka. Izmjereni su spektralni doppler indeksi poput indeksa pulsatilnosti (PI) i indeksa otpornosti (RI). Prikupljeni su uzorci krvi te su određene koncentracije testosterona, folikulostimulirajućeg hormona

(FSH) i luteinizirajućeg hormona (LH) u krvi. Rezultati su otkrili da se ehogenost testisa, repa epididimisa i akcesornih spolnih žlijezda tijekom sezone parenja promijenila. PI vrijednosti supratestikularne arterije (STA), marginalne arterije (MA), repa epididimisa, proširenja (ampulla), vezikularne žlijezde, pars disseminata prostate i bulbouretralne žlijezde bile su 0,85±0,04, 0,54±0,03, 0,4±0,03, 0,37±0,04, 0,51±0,03, 0,39±0,02 i 0,41±0,04. Nadalje, RI iznad navedenih kriterija bio je 0,51±0,04, 0,37±0,02, 0,3±0,03, 0,27±0,02, 0,31±0,03, 0,32±0,03, odnosno 0,32±0,03. Koncentracija testosterona u krvi bila je (4,78±0,46) ng/mL. Nadalje, FSH i LH bile su (3,71±0,43, odnosno 1,8±0,17) mIU/mL. Uočeno je da je testosteron vidno smanjio PI i RI vrijednosti akcesornih spolnih žlijezda rasplodnih mužjaka, što znači da je sezona parenja promijenila ehogenost testisa, epididimisa i akcesornih spolnih žlijezda. Testosteron je regulirao reproduktivne indekse (PI i RI) protoka krvi akcesornih spolnih žlijezda rasplodnih mužjaka. Rezulteti ove studije mogu se uzeti kao referentne vrijednosti egipatskih baladi jaraca tijekom sezone parenja da bi se poboljšala njihova reproduktivna učinkovitost.

Ključne riječi: *doppler, sezona, testisi, spolne žlijezde, rasplodni mužjaci*